

WHAT IS CLAIMED IS:

1 1. A Lac shuttle vector, comprising:

2 (a) a region which regulates a plasmid copy number,
3 wherein said region comprises an *E. coli* replication origin
4 sequence;

5 (b) an eukaryotic gene expression cassette, which
6 comprises an eukaryotic gene transcriptional promoter
7 sequence, a multiple cloning site and a transcriptional
8 terminator sequence, wherein a heterologous gene is inserted
9 into said multiple cloning site;

10 (c) a lactic acid bacteria plasmid sequence, which
11 comprises a plus origin of replication, and a nucleic acid
12 sequence encoding for a protein which relates to the lactic
13 acid bacteria plasmid replication; and

14 (d) a non-antibiotic resistance selection gene and the
15 promoter sequence thereof.

1 2. The Lac shuttle vector as claimed in claim 1,
2 wherein said eukaryotic gene transcriptional promoter is
3 cytomegalovirus (CMV) promoter.

1 3. The Lac shuttle vector as claimed in claim 1,
2 wherein said lactic acid bacteria plasmid sequence is the
3 plasmid of 2.1 kb size isolated from *Lactobacillus plantarum*.

4 4. The Lac shuttle vector as claimed in claim 3,
5 wherein the protein which relates to the lactic acid
6 bacteria plasmid replication is Rep A protein containing 317
7 amino acids.

1 5. The Lac shuttle vector as claimed in claim 1,
2 wherein said non-antibiotic resistance selection gene is β -
3 galactosidase gene.

1 6. The Lac shuttle vector as claimed in claim 5,
2 wherein the promoter of said β -galactosidase gene is
3 erythromycin resistance gene promoter.

1 7. The Lac shuttle vector as claimed in claim 1,
2 wherein the Lac Shuttle vector comprises the nucleotide
3 sequence set forth in SEQ ID NO:1 or a complementary
4 nucleotide sequence thereto, or a degenerate variant thereof.

1 8. The Lac shuttle vector as claimed in claim 1,
2 wherein the Lac Shuttle vector comprises the nucleotide
3 sequence set forth in SEQ ID NO:2 or a complementary
4 nucleotide sequence thereto, or a degenerate variant thereof.

1 9. The Lac shuttle vector as claimed in claim 1,
2 wherein the Lac Shuttle vector is selected from the group
3 consisting of:

4 (a) pCLP7 (having the configuration of restriction
5 sites in FIG.3, ATCC Accession No. PTA-2661); and

6 (b) pCLP8 (having the configuration of restriction
7 sites in FIG.3, ATCC Accession No. PTA-2663).

1 10. The Lac shuttle vector as claimed in claim 1,
2 wherein the host cell being transformed is a Gram-positive
3 bacterium, and the endogenous β -galactosidase gene of the

4 host cell is not capable of producing a normal enzymatic
5 function.

1 11. The Lac shuttle vector as claimed in anyone of
2 claim 10, wherein the host cell is the mutant of
3 *Lactobacillus casei* (subsp. *casei*), which is designated Ana-
4 1 (Lac⁻ mutant), (ATCC Accession No. PTA-2662).

1 12. A kit for expression of a heterologous gene,
2 comprising:
3 (a) the Lac shuttle vector as claimed in claim 1;
4 (b) a host cell which the endogenous β -galactosidase
5 gene thereof is not capable of producing a normal enzymatic
6 function; and
7 (c) an eukaryotic cell.

1 13. A DNA vaccine carrier comprising the Lac shuttle
2 vector as claimed in claim 1.

1 14. A method for selection of a host cell containing a
2 vector, comprising:
3 (i) introducing into said host cell the Lac shuttle
4 vector as claimed in claim 1,
5 wherein the endogenous β -galactosidase gene of said
6 host cell is not capable of producing a normal enzymatic
7 function; and
8 (ii) culturing said host cell transformed in step (i)
9 under conditions which lactose is the only carbon source.